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(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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(54) Title: MODIFIED OLIGONUCLEOTIDES HAVING IMPROVED ANTI-INFLUENZA ACTIVITY

(57) Abstract

The invention provides anti-influenza modified oligonucleotides that have greater efficacy in inhibiting influenza replication or propagation than previously described oligonucleotides. The greater efficacy arises from structural features such as chimeric or hybrid backbones, nuclease resistance-conferring terminal capping structures and/or self-complementary regions.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/03454

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/11 C07H21/04 C07H21/02 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 16902 (HYBRIDON, INC.) 14 November 1991 see page 3, line 16 - page 4, line 30 see claims	1-8, 17-28, 42,44
Y	WO,A,91 12323 (WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY) 22 August 1991 cited in the application see page 2, line 27 - page 3, line 14 see page 6, line 1 - line 18 see page 9, line 1 - line 24 see claims 1,4,5,10,12; table 2	1-8, 17-28, 42,44

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
9 January 1995	23.01.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Andres, S

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 03454 (ISIS PHARMACEUTICALS, INC.) 5 March 1992 cited in the application see page 5, line 14 - line 35 see page 8, line 33 - page 11 see claims	1-8,42
A	FEBS LETTERS, vol.232, no.1, May 1988, AMSTERDAM NL pages 96 - 98 ATABEKOV, K. ET AL. 'Site-specific enzymatic cleavage of TMV RNA directed by deoxyribo- and chimeric (deoxyribo-ribo)oligonucleotides' see the whole document	17,19, 20,22, 23,25, 26,28
A	EP,A,0 386 563 (BAYER A.G.) 12 September 1990 see claims	1
T	NUCLEIC ACIDS RESEARCH, vol.22, no.3, 11 February 1994, ARLINGTON, VIRGINIA US pages 453 - 456 ZHOU, L. ET AL. 'Synthesis of phosphorothioate-methylphosphonate oligonucleotide co-polymers' see the whole document	1

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03454

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see annex.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see annex

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-8 and 42(all part)
17-28,44

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Obscurities:
Claims 23 to 28 are not consistent with the claims they depend on. Indeed, claims 23 to 28 allow oligonucleotides with NO deoxyribonucleotide region. This is in clear contradiction with claim 17 (from which all other claims depend), which states that the antisense has to be an hybrid having a ribonucleotide region AND a deoxyribonucleotide region.

The search concerning claims 23 to 28 has nevertheless been done with the consideration that they should be read as having a deoxyribonucleotide region from about 1 to 100 deoxyribonucleotides.

This International Searching Authority found multiple inventions in this international application as follows:

1.- Claims: 1-8 and 42 (all partially)
Mixed phosphate backbone oligonucleotides having phosphorothioate and alkylphosphonate regions and their use as anti-influenza virus agents.

2.- Claims: 1-8 and 42 (all partially)
Mixed phosphate backbone oligonucleotides having phosphorodithioate and alkylphosphonate regions and their use as anti-influenza virus agents,

3.- Claims: 9-16 and 43
Mixed phosphate backboner oligonucleotides having phosphorothioate or phosphorodithioate and alkylphosphonothioate regions and their use as anti-influenza virus agents.

4.- Claims: 17-28 and 44
Modified oligonucleotides having deoxyribonucleotide and ribonucleotide regions and their use as anti-influenza virus agents.

5.- Claims 29 and 45
Modified oligonucleotides having at the 3' and/or 5' end a nuclease resistance conferring cap and their use as anti-influenza virus agents

6.- Claims: 30-41
Modified oligonucleotides having a self-complementary region and their use as anti-influenza virus agents.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/03454

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9116902	14-11-91	US-A-	5194428	16-03-93
		EP-A-	0527906	24-02-93
WO-A-9112323	22-08-91	US-A-	5149797	22-09-92
		EP-A-	0515511	02-12-92
		US-A-	5366878	22-11-94
		US-A-	5220007	15-06-93
WO-A-9203454	05-03-92	AU-B-	652577	01-09-94
		AU-A-	8536091	17-03-92
		CA-A-	2089562	15-02-92
		EP-A-	0543938	02-06-93
		JP-T-	6501843	03-03-94
EP-A-0386563	12-09-90	DE-A-	3907562	13-09-90
		DE-D-	59005494	01-06-94
		JP-A-	2276577	13-11-90

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Published
Without international search report and to be republished upon receipt of that report.

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MODIFIED OLIGONUCLEOTIDES HAVING IMPROVED ANTI-INFLUENZA ACTIVITY

This is a continuation-in-part of Ser. No. 07/909,069, filed July 2, 1992. This is also a continuation-in-part of Ser. No. 07/918,239, filed July 23, 1992. This is also a continuation-in-part of Ser. No. 07/698,568, filed May 10, 1991.

BACKGROUND OF THE INVENTION

Field Of The Invention

The invention relates to anti-sense oligonucleotides. More particularly, the invention relates to modified oligonucleotides that are capable of inhibiting replication or propagation of influenza virus.

Summary Of The Related Art

Influenza A virus is a membrane-enclosed virus whose genome is a segmented minus strand of RNA. The ten influenza virus genes are present on eight segments of the single-stranded RNA of strains A and B, and on seven segments of strain C. The segments are of varying sizes (ranging from 890 to 2341 nucleotides in length) and each is a template for synthesis for a different mRNA. The influenza virus virion contains virus-specific RNA polymerases necessary for mRNA synthesis from these templates and, in the absence of such

specific polymerases, the minus strand of influenza virus RNA is not infectious. Initiation of transcription of the mRNAs occurs when the influenza virus mRNA polymerase takes 12 to 15 nucleotides from the 5' end of a cellular mRNA or mRNA precursor and uses the borrowed oligonucleotide as a primer. Generally, the mRNAs made through this process encode only one protein. The M RNA and the NS RNA also encode spliced mRNAs, which results in production of two different proteins for each of these two segments.

Influenza viruses infect humans and animals (e.g., pigs, birds, horses) and may cause acute respiratory disease. There have been numerous attempts to produce vaccines effective against influenza virus. None, however, have been completely successful, particularly on a long-term basis. This may be due, at least in part, to the segmented characteristic of the influenza virus genome, which makes it possible, through reassortment of the segments, for numerous forms to exist. For example, it has been suggested that there could be an interchange of RNA segment between animal and human influenza viruses, which would result in the introduction of new antigenic subtypes into both populations. Thus, a long-term vaccination approach has failed, due to the emergence of new subtypes (antigenic "shift"). In addition, the surface proteins of the virus, hemagglutinin and neuraminidase, constantly undergo minor antigenic changes (antigenic

"drift"). This high degree of variation explains why specific immunity developed against a particular influenza virus does not establish protection against new variants. Hence, alternative antiviral strategies are needed. Although influenza B and C viruses cause less clinical disease than the A types, chemical antivirals should also be helpful in curbing infections caused by these agents.

Consequently, there is considerable interest in the development of anti-sense oligonucleotides that are capable of inhibiting the replication or propagation of influenza virus. Such anti-sense oligonucleotides hold the promise of providing broader protection against different strains of influenza, because they can be designed to hybridize to conserved regions of the influenza genome that are present in multiple strains of influenza virus.

Agrawal et al., U.S. Patent No. 5,194,428 the teachings of which are hereby incorporated by reference, discloses oligonucleotides having certain modified internucleotide linkages that are capable of inhibiting influenza replication by hybridizing to the influenza PB1 polymerase gene. Cowsert et al., WO92/03454 (1992) discloses antisense oligonucleotides that inhibit influenza virus propagation by hybridizing to the influenza polymerase 1, 2 or 3, or the hemagglutinin, nucleoprotein, neuraminidase, matrix protein, or nonstructural

protein gene, or to various splice junction sites or packaging sequences.

Pederson et al., U.S. Patent Nos. 5,149,797 and 5,XXX,XXX (Serial No. 07/839,472, allowed December 24, 1992), the teachings of which are hereby incorporated by reference, discloses chimeric mixed phosphate backbone oligonucleotides having RNase H activating segments adjacent to RNase H inactivating segments.

Thus, anti-sense oligonucleotides show promise as anti-influenza therapeutic agents. As with any such agents, however, there remains a need for improved agents that have even greater efficacy in inhibiting influenza virus replication or propagation. Such improved anti-sense oligonucleotides would be useful for studying which regions of the influenza virus genome are the best candidates for broad cross-strain inhibition as well as for use, and as anti-influenza therapeutic agents.

BRIEF SUMMARY OF THE INVENTION

The invention provides modified oligonucleotides having greater efficacy in inhibiting the replication or propagation of influenza virus than previously known oligonucleotides. These modified oligonucleotides are characterized by having a nucleotide sequence sufficiently complementary to an essential nucleic acid of influenza virus origin to hybridize to such influenza virus nucleic acid in a cell. In preferred embodiments, the essential nucleic acid is a portion of the PB1, 2 or 3 polymerase gene or to the hemagglutinin, nucleoprotein, neuraminidase, matrix protein, or nonstructural protein gene of influenza or to the various influenza splice junction sites or packaging sequences.

In various embodiments, modified oligonucleotides according to the invention have one or more type of modified internucleotide linkage. In a preferred embodiment, at least some of the modified internucleotide linkages are phosphorothioate or phosphorodithioate linkages. In certain preferred embodiments, phosphorothioate internucleotide linkages are present in a modified oligonucleotide that also contains other modified, i.e. nonphosphodiester, linkages. Preferably these other modified internucleotide linkages are alkylphosphonate or alkylphosphonothioate linkages. Most preferably, these other modified internucleotide linkages are present at or near one or both ends of the oligonucleotide.

In additional preferred embodiments of modified oligonucleotides according to the invention, phosphorothioate or phosphorodithioate internucleotide linkages are present in an oligonucleotide having one or more modified nucleoside. Preferably, such modified nucleoside is a 2'-O alkyl nucleoside. Most preferably, at least some modified nucleosides are present at or near one or both ends of the oligonucleotide.

In yet additional preferred embodiments of modified oligonucleotides according to the invention, phosphorothioate or phosphorodithioate internucleotide linkages are present in an oligonucleotide having an exonuclease resistance-conferring cap structure at one or both ends. Preferably, such a cap structure is present at least at the 3' end of the molecule. Such cap structures may also be present at one or both ends of all embodiments of modified oligonucleotides according to the invention and preferably are present at least at the 3' end of the oligonucleotide.

In further preferred embodiments of modified oligonucleotides according to the invention, phosphorothioate or phosphorodithioate internucleotide linkages are present in an oligonucleotide that is self-stabilized by having a self-complementary region involving nucleotides at or near one or both ends, and preferably at least at or near the 3' end, whereby the oligonucleotide forms a hairpin-like structure.

Each of these modified oligonucleotides according to the invention provides greater efficacy in inhibiting replication or propagation of influenza than any known oligonucleotide or modified oligonucleotide that hybridizes to the same gene. Each of these modified oligonucleotides according to the invention may optionally also contain other modifications to the sugars or bases of the oligonucleotide and may also optionally have additional internucleotide linkages other than phosphorothioate, phosphorodithioate, alkylphosphonate or alkylphosphonothioate linkages.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence of an influenza virus PB1 (polymerase 1) gene, against which complementary antisense oligonucleotides can be prepared.

Figure 2 shows a self-stabilized anti-influenza modified oligonucleotide according to the invention.

Figure 3 shows an alternative form of a self-stabilized anti-influenza modified oligonucleotide according to the invention.

Figure 4 shows certain preferred cap structures that confer exonuclease resistance upon oligonucleotides.

Figure 5 shows <u>in vivo</u> nucleolytic degradation patterns for 5'-capped, 3'-capped and uncapped oligonucleotides.

Figure 6 shows DNA Polymerase I 3'-exonuclease degradation patterns for self-stabilized and non-self-stabilized oligodeoxynucleotide phosphodiesters.

Figure 7 shows DNA Polymerase I 3'-exonuclease degradation patterns for self-stabilized and non-self-stabilized oligodeoxynucleotide phosphorothioates.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to anti-sense oligonucleotides having anti-influenza activity. Modified oligonuclestides having _in vivo_ activity against influenza are referred to herein as anti-influenza modified oligonucleotides. The invention provides anti-influenza modified oligonucleotides that have greater efficacy in inhibiting replication or propagation of influenza virus than known oligonucleotides or modified oligonucleotides that hybridize to the same gene. Modified oligonucleotides according to the invention have specific preferred characteristics that are discussed in greater detail for each preferred embodiment below. In addition to these characteristics, modified oligonucleotides according to the invention may optionally have additional ribonucleotide, 2'-substituted ribonucleotide, and/or deoxyribonucleotide monomers, any of which are connected together via 5' to 3' linkages which may include any of the internucleotide linkages known in the art. Preferably, such modified oligonucleotides may optionally contain phosphodiester, phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate and/or sulfone internucleotide linkages. Those skilled in the art will

recognize that the synthesis of oligonucleotides containing any of these internucleotide linkages is well known to those skilled in the art, as is illustrated by articles by Uhlmann and Peyman, Chemical Reviews 90:543-584 (1990) and Schneider and Banner, Tetrahedron Lett. 31:335 (1990). Preferably, modified oligonucleotides according to the invention should contain from about 6 to about 100 monomers in total. Such modified oligonucleotides may also optionally contain modified nucleic acid bases and/or sugars, as well as added substituents, such as diamines, cholesteryl or other lipophilic groups.

Various preferred embodiments of modified oligonucleotides according to the invention are illustrated in Table I, below. Although these embodiments all have a nucleotide sequence from the same region of the influenza PB1 polymerase gene, those skilled in the art will recognize that the anti-influenza efficacy of oligonucleotides having nucleotide sequences complementary to other essential nucleic acid sequences of influenza virus can also be enhanced by incorporating into such oligonucleotides the structural features of preferred embodiments of modified oligonucleotides according to the invention. For purposes of the invention, complementary means having a sequence that hybridizes to the essential nucleic acid sequence under physiological conditions. An essential nucleic acid sequence of influenza

virus means a nucleic acid sequence that is required for replication or propagation of influenza virus. Such essential nucleic acid sequences can be from any known strain of influenza virus. For example such oligonucleotides can have other sequences from the influenza PB1 polymerase gene (polymerase 1), as shown in Table I of U.S. Patent No. 5,194,428, the teachings of which are hereby incorporated by reference. Indeed, any sequence from the influenza PB1 (polymerase 1) gene [SEQ. ID. No. 1] (see Figure 1) should serve as the basis for modified oligonucleotides according to the invention. Alternatively, sequences from other influenza sequences or genes can be used (see Table II). As a practical matter, the structural features of preferred embodiments of modified oligonucleotides according to the invention should enhance the anti-influenza activity of any antisense oligonucleotide having a nucleotide sequence that hybridizes in a cell with any essential nucleic acid sequence of influenza virus.

Each preferred embodiment of modified oligonucleotides according to the invention is separately discussed in greater detail below.

TABLE I

Modified Oligonucleotides Having Superior Anti-Influenza Activity

Compound Identification NO.]	Sequence and Structure	[SEQ. ID
CMPD A 2]	CAGAGCAAAATCATCAGAAGA[1]	[SEQ. ID NO.
CMPD B 3]	CAGAGCAAAATCATCAGAAGA[2]	[SEQ. ID NO.
CMPD C 4]	CAGAGCAAAATCATCAGAAGA[3]	[SEQ. ID NO.
CMPD D 5]	CAGAGCAAAATCATCAGAAGA[3]	[SEQ. ID NO.
CMPD E 6]	CAGAGCAAAATCATCAGAAGA[2]	[SEQ. ID NO.
CMPD F 7]	CAGAGCAAAATCATCAGAAGA[2]	[SEQ. ID NO.
CMPD G 8]	CAGAGCAAAATCATCAGAAGA[3]	[SEQ. ID NO.
CMPD H 9]	CAGAGCAAAATCATCAGAAGA[3]	[SEQ. ID NO.
CMPD I 10]	CAGAGCAAAATCATCAGAAGA-C_{12}[4]	[SEQ. ID NO.
CMPD J 12]	AGAGCAAAATCATCAGAAG[3]	[SEQ. ID NO.
CMPD K 14]	GAGCAAAATCATCAGAA[3]	[SEQ. ID NO.
CMPD L 15]	CAGAGCAAAATCATCAGAAGA[3]	[SEQ. ID NO.
CMPD M 16]	CAGAGCAAAATCATCAGAAGATTCTGATGA[5]	[SEQ. ID NO.

0 All sequences shown 5' to 3'; nonunderlined regions are oligonucleotide phosphorothioates.

1 Underscoring represents nucleotides connected by methylphosphonate internucleotide linkage.

2 Underscoring represents nucleotides connected by methylphosphonothioate linkage.

3 Underscoring represents 2'-OMe nucleotides.

4 C_{12} represents cap structure.

5 Self-stabilized.

TABLE II
Additional Anti-Influenza Oligonucleotide Potential Sequences

Sequence	Target	[SEQ. ID. No.]
CTT TCC ATA TTG AAT ATA AT	AUG segment 1 polymerase 3	[SEQ. ID. No. 17]
ACA TCC ATT CAA ATG GTT TG	AUG segment 2 polymerase 1	[SEQ. ID. No. 18]
TCT TCC ATT TTG GAT CAG TA	AUG segment 3 polymerase 2	[SEQ. ID. No. 19]
GCC TTC ATT TTG GTT GTT TT	AUG segment 4 hemagglutinin	[SEQ. ID. No. 20]
GAC GCC ATG ATT TTG ATG TC	AUG segment 5 nucleoprotein	[SEQ. ID. No. 21]
GGA TTC ATT TTA AAC CCC TG	AUG segment 6 neuraminidase	[SEQ. ID. No. 22]
AGA CTC ATC TTT CAA TAT CT	AUG segment 7 matrix protein	[SEQ. ID. No. 23]
GAT AGA GAG AAC GTA CGT TT	left splice junction segment 7	[SEQ. ID. No. 24]
TCT GAT AGG CCT GCA AAT TT	right splice junction segment 7	[SEQ. ID. No. 25]
GGA TCC ATT ATG TCT TTG TC	AUG segment 8 nonstructural protein	[SEQ. ID. No. 26]
CAT GTC GGT TAG GTA ACG CG	splice branch segment 8	[SEQ. ID. No. 27]
GCA ATC TAC CTG AAA GCT TG	right splice junction segment 8	[SEQ. ID. No. 28]
AGC AGT ATG TCC TGG AAG AG	left splice junction segment 8	[SEQ. ID. No. 29]
AAA ACG ACC TTG TTT CTA CT	packaging sequence segment 1	[SEQ. ID. No. 30]
AAA AAT GCC TTG TTC CTA CT	packaging sequence segment 2	[SEQ. ID. No. 31]

AAA AGT ACC TTG TTT CTA CT	packaging sequence segment 3 [SEQ. ID. No. 32]
AAA ACA CCC TTG TTT CTA CT	packaging sequence segment 4 [SEQ. ID. No. 33]
AAA ATA CCC TTG TTT CTA CT	packaging sequenc segment 5 [SEQ. ID. No. 34]
AAA AAC TCC TTG TTT CTA CT	packaging sequence segment 6 [SEQ. ID. No. 35]
AAA ACT ACC TTG TTT CTA CT	packaging sequence segment 7 [SEQ. ID. No. 36]
AAA ACA CCC TTG TTT CTA CT	packaging sequence segment 8 [SEQ. ID. No. 37]

In a first preferred embodiment, anti-influenza modified oligonucleotides according to the invention are in the form of a mixed backbone chimeric oligonucleotide having one or more regions of nucleotides connected by phosphorothioate or phosphorodithioate internucleotide linkages ("phosphorothioate or phosphorodithioate region") as well as one or more regions of nucleotides connected by alkylphosphonate internucleotide linkages ("alkylphosphonate region"). In this embodiment, at least one alkylphosphonate region preferably includes nucleotides at or near the 5' end and/or the 3' end of the oligonucleotide. For purposes of the invention, "at or near the 5' or the 3' end of the oligonucleotide" means involving at least one nucleotide within about 5 nucleotides from the 5' or 3' end of the oligonucleotide. Preferably, the alkylphosphonate region comprises from about 2 to about 10

contiguous nucleotides connected by alkylphosphonate linkages. Preferably, the phosphorothioate or phosphorodithioate region comprises at least 3, and up to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages. An example of an anti-influenza modified oligonucleotide according to this embodiment of the invention is shown in Table I as CMPD A.

Anti-influenza modified oligonucleotides according to this embodiment of the invention are synthesized by solid phase methods, alternating H-phosphonate chemistry and sulfur oxidation for phosphorothioate regions, and alkylphosphonamidate chemistry for alkylphosphonate regions. A preferred H-phosphonate approach is taught by Agrawal et al., U.S. Patent No. 5,149,798, the teachings of which are hereby incorporated by reference. Alkylphosphonamidite chemistry is well known in the art, as illustrated by Agrawal and Goodchild, Tetrahedron Lett. 28:3539-3542 (1987). Synthesis of phosphorodithioate-containing oligonucleotides is also well known in the art, as illustrated by U.S. Patent No. 5,151,510, the teachings of which are hereby incorporated by reference (See also, e.g., Marshall and Caruthers, Science 259: 1564-1570 (1993) and references cited therein).

In a second preferred embodiment, anti-influenza modified oligonucleotides according to the invention are in the form of a mixed backbone chimeric oligonucleotide having one or more

region of nucleotides connected by phosphorothioate or phosphorodithioate internucleotide linkages ("phosphorothioate or phosphorodithioate region"), as well as one or more region of nucleotides connected by alkylphosphonothioate or arylphosphonothioate internucleotide linkages ("alkylphosphonothioate region"). In this embodiment, at least one alkylphosphonothioate region preferably includes nucleotides at or near the 5' end and/or the 3' end of the oligonucleotide. Preferably, the alkylphosphonothioate region comprises from about 2 to about 10 contiguous nucleotides connected by alkylphosphonothioate linkages. Preferably, the phosphorothioate or phosphorodithioate region comprises at least 3, and up to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages. Examples of anti-influenza modified oligonucleotides according to this embodiment of the invention are shown in Table I as CMPD B, CMPD E and CMPD F.

Anti-influenza modified oligonucleotides according to this embodiment of the invention are synthesized by solid phase methods, alternating chemistries for each region to be synthesized. Phosphorothioate or phosphorodithioate regions are synthesized as described for the first embodiment. Alkylphosphonothioate regions are synthesized by coupling together two or more nucleosides via alkylphosphite linkages, then oxidatively thiolating the alkylphosphite linkages to

produce alkylphosphonothioate linkages. This synthesis procedure is set forth in detail in Example 1.

In a third preferred embodiment, anti-influenza modified oligonucleotides according to the invention are in the form of a hybrid oligonucleotide having regions of deoxyribonucleotides ("deoxyribonucleotide regions") and regions of ribonucleotides or 2'-substituted ribonucleotides ("ribonucleotide regions"). Preferably, from about one to about all of the internucleotide linkages are phosphorothioate or phosphorodithioate linkages. Preferred 2'-substituted ribonucleotides are halo, amino, alkyl, aryl or lower alkyl (1-6 carbon atoms) substituted ribonucleotides, especially 2'-OMe-ribonucleotides. Preferably, at least some of the ribonucleotide regions include nucleotides present at or near the 5' end and/or the 3' end of the oligonucleotide. Most preferably, the ribonucleotide regions each comprise from about 2 and preferably from about 4 to about 100 contiguous ribonucleotides and/or 2'-substitute oligonucleotides. The deoxyribonucleotide regions are optional, and when present may contain from about 1 to about 100 contiguous deoxyribonucleotides. Examples of anti-influenza modified oligonucleotides according to this embodiment of the invention are shown in Table I as CMPD C, CMPD D, CMPD G, CMPD H, CMPD K, CMPD M and CMPD N.

Anti-influenza modified oligonucleotides according to this embodiment of the invention are typically synthesized by solid phase methods, preferably by the H-phosphonate approach, using deoxynucleotide H-phosphonates for deoxyribonucleotide regions, and ribonucleotide or 2'-substituted ribonucleotide H-phosphonates for ribonucleotide regions.

In a fourth preferred embodiment, anti-influenza modified oligonucleotides according to the invention are in the form of an oligonucleotide having at its 5' and/or 3' end a cap structure that confers exonuclease resistance to the oligonucleotide. Such modified oligonucleotides preferably also have from 1 to about all modified (non-phosphodiester) internucleotide linkage. Preferred cap structures include those shown in Figure 4, as well as lower alkyl (C1-C12) or alcohol groups. Preferred modified internucleotide linkages include phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, sulfone, phosphorothioate and phosphorodithioate linkages.

Anti-influenza modified oligonucleotides according to this embodiment of the invention are synthesized according to procedures well known in the art (see e.g., Uhlmann and Peyman, Chemical Reviews 90:543-584 (1990); Schneider and Banner, Tetrahedron Lett. 31:335 (1990)). For

oligonucleotides having cap structures at the 3' end, the cap structure is reversibly attached to the solid support and is then coupled to the first nucleotide monomer in the synthesis scheme. For oligonucleotides having cap structures at the 5' end, the cap structure is coupled to the end of the oligonucleotide after addition of the last nucleotide monomer in the synthesis scheme.

In a fifth embodiment, anti-influenza modified oligonucleotides are self-stabilized by having a self-complementary region that hybridizes intramolecularly with the oligonucleotide to form an exonuclease resistant hairpin-like structure. Anti-influenza modified oligonucleotides according to this embodiment of the invention are generally characterized by having two regions: an influenza hybridizing region and a self-complementary region. The influenza hybridizing region has a nucleotide sequence that is complementary to an essential nucleic acid sequence of influenza virus. Preferably, this region has from about 6 to about 100 nucleotides. One form of this embodiment of the invention is shown in Figure 2. In this form, the influenza hybridizing region is shown as connected rectangular squares, and the self-complementary region is shown as connected circles. The complementary nucleic acid sequence in a target influenza messenger RNA molecule is represented by connected diamonds. Hydrogen bonding between nucleotides is indicated

by dots. The oligonucleotide is stabilized, i.e., rendered resistant to exonucleolytic degradation by base-pairing between the target hybridizing region and the self-complementary region and/or by base-pairing between complementary sequences within the self-complementary region. When the oligonucleotide encounters an influenza nucleic acid molecule having a complementary nucleic acid sequence, base-pairing between the influenza hybridizing region and the self-complementary region of the oligonucleotide is disrupted and replaced by base-pairing between the influenza hybridizing region of the oligonucleotide and the complementary nucleic acid sequence of the nucleic acid molecule. This disruption and replacement of base-pairing takes place because the intermolecular base-paired structure formed by the hybrid between the target nucleic acid sequence and the target hybridizing region is more thermodynamically stable than the intramolecular base-paired structure formed by the self-complementary oligonucleotide.

A second form of an oligonucleotide according to this embodiment of the invention operates in a similar way as the first form, but forms a different structure upon self-complementary base-pairing. This alternative form forms a hammer-like structure as shown in Figure 3. In this form, the self-complementary region contains oligonucleotide sequences that can base pair with other oligonucleotide sequences within

the self-complementary region. The self-complementary region may also contain oligonucleotide sequences that are complementary to the influenza hybridizing region.

The second significant region of self-stabilized oligonucleotides according to the invention is the self-complementary region. The self-complementary region contains oligonucleotide sequences that are complementary to other oligonucleotide sequences within the oligonucleotide. These other oligonucleotide sequences may be within the influenza hybridizing region or within the self-complementary region, or they may span both regions. The complementary sequences form base pairs, resulting in the formation of a hairpin structure, as shown in Figure 2, or a hammer-like structure, as shown in Figure 3. Either the hairpin structure or the hammer-like structure can have loops resulting from non-base-paired nucleotides, as shown in Figure 2 for the hairpin structure, or can be devoid of such loops, as shown in Figure 3 for the hammer-like structure. The number of base-pairs to be formed by intra-molecular hybridization involving the self-complementary region may vary, but should be adequate to maintain a double-stranded structure so that the 3' end is not accessible to endonucleases. Generally, about 4 or more base-pairs will be necessary to maintain such a double-stranded structure. In a preferred embodiment, there are about 10 intramolecular base-pairs formed in the self-stabilized

oligonucleotide, with the 10 base pairs being consecutive and involving the 3'-most nucleotides. Of course, the intra-molecular base-pairing can be so extensive as to involve every nucleotide of the oligonucleotide. Preferably, this will involve a self-complementary region of about 50 nucleotides or less.

Oligonucleotides according to this embodiment may have from 1 to about all modified internucleotide linkages, as described for the fourth embodiment. Preferably, at least either the influenza hybridizing region or the self-complementary region, and most preferably both, will contain from about 2 to about all nucleotides being coupled by phosphorothioate and/or phosphorodithioate linkages.

An example of an anti-influenza modified oligonucleotide according to this embodiment of the invention is shown in Table I as CMPD O.

Those skilled in the art will recognize that the features of the various preferred embodiments described above can be combined to produce additional embodiments that may have even greater anti-influenza activity. Thus, the invention contemplates anti-influenza modified oligonucleotides having every possible combination of chimeric features, hybrid features, cap structures and self-stabilizing character, all as described herein.

Each of the preferred embodiments of the present invention has greater ability to inhibit influenza replication or propagation than anti-sense oligonucleotides of the prior art that hybridize to the same influenza mRNA. For example, U.S. Patent No. 5,194,428, teaches modified oligonucleotides that inhibit influenza virus replication. Among those modified oligonucleotides are oligonucleotide phosphorothioates that hybridize to the influenza PB1 (polymerase 1) gene. In the present study, an oligonucleotide phosphorothioate that hybridizes to the influenza PB1 polymerase gene was tested for its ability to inhibit influenza virus replication in comparison with various preferred embodiments of the present invention. Each embodiment of the present invention tested demonstrated surprisingly improved efficacy in anti-influenza activity, relative to the oligonucleotide phosphorothioate that binds to the same site on the same gene. As shown in Table III, below, anti-influenza modified oligonucleotides according to the invention gave a reduction in the 50 per cent inhibitory concentration (IC_{50}) ranging from 2 to nearly fifteen fold.

TABLE III

Anti-Influenza Activity Of Oligonucleotide

Oligonucleotide Improvement	IC_{50} (µg/ml)	X-Fold
CAGAGCAAAATCATCAGAAGA[1]	317[2]	-
CMPD A	N.T.	-
CMPD B	34	9.3
CMPD C	55	5.8
CMPD D	58[2]	5.5
CMPD E	N.T.	-
CMPD F	178	1.8
CMPD G	147	2.2
CMPD H	59	5.4
CMPD I	45	7.0
CMPD J	27[2]	11.7
CMPD K	22[2]	14.4
CMPD L	61	5.2
CMPD M	175	1.8
CMPD N	104[2]	3.0
CMPD O	23[2]	13.8

[1] oligodeoxynucleotide phosphorothioate control

[2] average of two experiments

Such oligonucleotides are useful for a variety of purposes. First, they can be used in studies to determine which influenza genes and sites within such genes provide the best basis for anti-sense oligonucleotides having broad effectiveness against multiple strains of influenza virus. Second, they can be used to determine what structural features

or combination of structural features provide the greatest effectiveness against influenza *in vitro* and *in vivo*. Finally, such oligonucleotides are useful as therapeutic agents for treating influenza infections. For such treatment, oligonucleotides may be administered intraperitoneally, intranasally, orally or anally. Preferably, such oligonucleotides will be administered at a concentration of from about 1 to about 50 mg/kg body weight.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not intended to be limiting in nature.

EXAMPLE 1

Synthesis Of A Chimeric Oligonucleotide Having Methylphosphonate And Phosphorothioate Regions

Chimeric oligonucleotides having methylphosphonate and phosphorothioate regions were synthesized using methylphosphonamindites for methylphosphonate regions and nucleoside H-phosphonates for phosphorothioate regions.

Methylphosphonamidite synthesis was as follows:

Methylchloro-N, N-diisopropylaminosphophine was prepared by reaction of methyldichlorophosphine (51 mmol) with diisopropylamine (102 mmol) in ether at 15° under nitrogen. After removal of salt by filtration and evaporation of solvent, methylchloro-N_1N-diisopropylaminophosphine was obtained as an oil (48 mmol, 95% of theory) that was

characterized by ^{1}H and ^{31}P NMR and was stable at -20° for at least eight weeks. The product was reacted with the usual protected nucleosides in dichloromethane containing N, N-diisopropylethylamine at room temperature for 10-20 min. Aqueous work up and precipitation from ethyl acetate using pentane at -30° to -40° gave product as white solids in 80-90% yield. Products were pure by tlc on silica in CH_2Cl_2:EtOAc:Et^3N(9:9:2) and were characterized by ^{1}H and ^{31}P NMR.

These products were used in automated DNA synthesizer using the same conditions and program used for standard phosphoramidite reagents. Nucleotides were dissolved in acetonitile at a concentration of 33 mg/ml and activated with tetrazole. Synthesis on prepacked CPG support was performed using a coupling time of 1 minute.

Coupling efficiency was followed by the dmethoxytrityl assay and was found to be the same as for control syntheses run in parallel using phosphoramidites.

After coupling, the product was detriylated then cleaved from the support with NH_4OH at room temperature for 2 hrs and deblocked using ethylenediamine: ethanol (1:1) at room temperature for 4 hrs. This basic treatment caused about 1% degradation of the internucldoside phosphonate group in a model study assayed by HPLC.

H-phosphonate synthesis was as exemplified in U.S. Patent No. 5,149,798, the teachings of which are hereby incorporated by reference. Then, the H-phosphonates were converted to phosphorthioates by oxidation with 0.2 M sulfur in carbon disulfide/pyridine/triethylamine (9:9:1 vol/vol).

EXAMPLE 2

Synthesis Of A Chimeric Oligonucleotide Having Methylphosphonothioate And Phosphorothioate Regions

For preparing a modified oligonucleotide having regions of four contiguous nucleotide methylphosphonothioates at either end and a region of 12 contiguous oligonucleotide phosphorothioates in the middle, the following procedure on 8 micromole scale was used. A first monomer was pre-bound to a control pore glass (CPG) solid support. A second monomer, which was a deoxynucleotide methylphosphonamidite, was coupled to the first monomer using a standard amidite coupling cycle (see e.g., Agrawal and Goodchild, Tetrahedron Lett. 28:3539-3542 (1987)). In separate cycles, three more deoxynucleotide methylphoshonamidites were sequentially coupled to the growing chain. Then oxidative thiolation was carried out, using 1% Beaucage reagent (3H-1,2-benzodithiole-2-one) in acetonitrile for 5 minutes at ambient temperature to generate a CPG-bound pentanucleotide methylphosphonothioate. The next 12 monomers were added sequentially by the H-phosphonate approach of Agrawal and Zamecnik, U.S. Patent No. 5,149,798, the teachings

of which are hereby incorporated by reference. Then, the H-phosphonates were converted to phosphorothioates by oxidation with 0.2 M sulfur in carbon disulfide/pyridine/triethylamine (9:9:1 vol/vol). The final four monomers, which were deoxynucleotide methylphosphonothioates, were added, then oxidatively thiolated as described above. The resulting oligonucleotide was deprotected at room temperature for 30 minutes in 0.5 ml ethylene diamine, then kept at room temperature for 6 hours with occasional stirring. Finally, the mixture was filtered and evaporated in vacuo to obtain a solid mass and the mass was dissolved in water and desalted on Sep Pak C_{18}.

EXAMPLE 3

Resistance Of Oligonucleotides Having Methylphosphonothioate Linkages To Nucleolytic Degradation

Oligonucleotides having 2-4 contiguous deoxynucleotide methylphosphonates at 3' ends and otherwise having all deoxynucleotide phosphodiesters were tested for their relative resistance to 3' exonucleolytic degradation compared with an oligonucleotide phosphodiester. For each oligonucleotide, 0.4 A_{260} units of oligonucleotide was lyophilized, dissolved in 0.5 ml buffer (10 mM Tris, 10 mM $MgCl_2$, pH 8.5) and mixed with 5 µl (1.5 milliunits) of snake venom phosphodiesterase. The mixture was incubated at 37°C in a thermally regulated cell

and A_{260} was plotted against time. Increase in hyperchromicity was used as the indicator for oligonucleotide degradation. The results are shown in Table IV, below.

These results demonstrate that oligonucleotides having methylphosphonothioate linkages near the 3' end were far more stable than the oligonucleotide lacking such linkages. In addition, oligonucleotide stability increased with increasing numbers of methylphosphonothioate linkages (4 linkages>>3 linkages> >2 linkages).

TABLE IV
Resistance Of Oligonucleotides To Nucleolytic Degradation

Oligonucleotide	t 1/2 (seconds)	% increase in hyperchromicity
Oligonucleotide phosphodiester	44	22.56
Oligonucleotide with 2 3' terminal deoxynucleotide methylphosphonothioates	210	24.58
Oligonucleotide with 3 3' terminal deoxynucleotide methylphosphonothioates	264	18
Oligonucleotide with 4 3' terminal deoxynucleotide methylphosphonothioates	401	15.54

EXAMPLE 4

Synthesis Of A Hybrid Oligonucleotide Phosphorothioate Having Deoxyribonucleotide And 2'-OMe-Ribonucleotide Regions

Hybrid oligonucleotide phosphorothioates were synthesized on CPG on a 5-6 μmole scale on an automated synthesizer (model 8700, Millipore, Milford, MA) using the H-phosphonate approach described in U.S. Patent No. 5,149,798, the teachings of which are hereby incorporated by reference.

Deoxyribonucleoside H-phosphonates were obtained from Millipore. 2'-OMe ribonucleotide H-phosphonates were synthesized by standard procedures. Segments of oligonucleotides containing 2'-OMe nucleoside were assembled by using 2'-OMe ribonucleoside H-phosphonates for the desired cycles. Similarly, segments of oligonucleotides containing deoxyribonucleosides were assembled by using deoxynucleoside H-phosphonates for the desired cycles. After assembly, CPG bound oligonucleotide H-phosphonate was oxidized with sulfur as described in Example 2 to generate the phosphorothioate linkage. Oligonucleotides were then deprotected in concentrated NH_4OH at 40°C for 48 hours.

Crude oligonucleotide (about 500 A_{260} units) was analyzed on reverse low pressure chromatography on a C_{18} reversed phase medium. The DMT group was removed by treatment with 80% aqueous acetic acid, then the oligonucleotides were dialyzed against distilled water and lyophilized.

EXAMPLE 5

Relative Nuclease Resistance Of Hybrid Oligonucleotide Phosphorothioates

To test the relative nuclease resistance of various hybrid oligonucleotide phosphorothioates, the oligonucleotides were treated with snake venom phosphodiesterase (SVPD). About 0.2 A_{260} units of oligos having no 2'-OMe-RNA region, or having 3 and 4 contiguous 2'-OMe ribonucleotides at 5' and 3' ends, respectively, or having all 2'-OMe nucleotides, were dissolved in 500 µl buffer (40 mM NH_4CO_3, pH 4.0 + 20 mM $MgCl_2$), and mixed with 0.1 units SVPD. The mixture was incubated at 37°C for 420 minutes. After 0, 200 and 420 minutes, 165 µl aliquots were removed and analyzed using ion exchange HPLC. The oligonucleotide having all 2'-OMe-ribonucleotides was very resistant to SVPD, whereas the oligonucleotide having no 2'-OMe-ribonucleotides was digested almost to completion and the oligonucleotide having 5' and 3' terminal 2'-OMe-RNA regions was digested to 50%. An oligonucleotide phosphodiester was digested to about 80% in one minute using one tenth of the concentration of SVPD.

These results indicate that the presence of 2'-OMe ribonucleotides in an oligonucleotide phosphorothioate enhances resistance to exonucleolytic digestion and that this enhanced resistance increases when a larger proportion of 2'-OMe ribonucleotides are used. Due to the similar character

and behavior of ribonucleotides, other 2'-substituted ribonucleotides and 2'-OMe ribonucleotides, these results also suggest that similar enhancement of nuclease resistance would be obtained for hybrid oligonucleotide phosphorothioates and/or phosphorodithioates having ribonucleotides, 2'-substituted ribonucleotides, or a mixture of ribonucleotides and 2'-substituted ribonucleotides.

EXAMPLE 6

Synthesis Of An Oligonucleotide Phosphorothioate Having A 3' Cap Structure

Oligonucleoside phosphorothioates were synthesized on a Model 8700 automated synthesizer (Milligen-Biosearch, Burlington, MA) using H-phosphonate chemistry on controlled pore glass (CPG), followed by oxidation with 0.2 M sulfur in carbon disulfide/pyridine/triethylamine (9:9:1 vol/vol). Synthesis was carried out on a 5-10 micromolar scale. Oligonucleoside phosphorothioates were purified by low pressure ion exchange chromatography (DEAE-cellulose, DE-50 Whatman), followed by reverse phase chromatography (C_{18}) and dialysis. 5'-capped oligonucleoside phosphorothioates were prepared by carrying out the last coupling, after the assembly of the required sequence, with N-Fmoc-O'-DMTr-3-amino-1,2-propanediol-H-phosphonate. The 5'-capped oligonucleoside H-phosphonate was then oxidized with sulfur. 3'-capped oligonucleoside phosphorothioates were assembled on N-Fmoc-O'-

DMTr-3-amino-1,2-propanediol-CPG, followed by sulfur oxidation. Combination of these procedures was used to produce 3',5'-capped oligonucleoside phosphorothioates.

Alternatively, oligonucleoside phosphorothioates having other 3' or 5' cap structures, (see e.g., Figure 4), are prepared by substituting the phosphonate or CPG-derivatized cap structures for the N-Fmoc-O'-DMTr-3-amino-1,2-propanediol-H phosphonate or CPG in the capping procedure. Similarly, capped modified oligonucleotides other than oligonucleotide phosphorothioates are prepared in an analogous manner by appending the capping procedure to the appropriate synthetic procedure.

EXAMPLE 7

In Vivo Stability Of Oligonucleotide Phosphorothioates Having Terminal Cap Structures

Male CDC2F1 mice (average weight 20 grams) were treated by intravenous or intraperitoneal injection with a 30 mg/kg dose of radiolabelled oligonucleotides dissolved in 200 microlitres physiological saline. Each capped or uncapped oligonucleotide was administered to three mice. Urine was collected separately from each animal up to 24 hours post-dosing, then extracted with proteinase K. (2 mg/ml, final concentration) in 0.5% SDS, 10 mM 20 mM Tris Cl (pH 7.6), 10 mM EDTA for one hour at 37°C, followed by phenol-chloroform extraction and ethanol precipitation. Recovered

oligonucleotides were then analyzed by PAGE (20% polyacrylamide/7 M urea) followed by autoradiography. Radioactivity was also measure from cage rinse to account for urine spill.

Twenty-four hours after dosing, about 30% of oligonucleoside phosphorothioates were excreted, whether capped or uncapped. Excreted uncapped and 5'-capped oligonucleoside phosphorothioates were extensively degraded, as shown in Figure 5. Excreted 3'-capped and 3',5'-capped oligonucleoside phosphorothioates, in contrast, demonstrated virtually no degradation. This indicates that in vivo degradation of oligonucleoside phosphorothioates excreted in urine is mediated by 3'-exonuclease activity which can be inhibited by adding a cap to the 3' hydroxyl group of the oligonucleotide.

EXAMPLE 8

Nuclease Resistance Of Self-Stabilized Oligonucleotide Phosphodiesters

The control oligonucleotide used for this study was an oligodeoxynucleotide phosphodiester without a self-complementary region. The test compound was identical, except that it had a 3' self complementary region of 10 nucleotides. To control for any size effects, another control oligodeoxynucleotide phosphodiester was used that was

identical to the first control oligonucleotide except for having at its 3' end 10 mismatched nucleotides (T_{10}).

The oligonucleotides were tested for their relative resistance to 3' exonucleolytic degradation. For each oligonucleotide, 0.4 A_{260} units of oligonucleotide was lyophilized, dissolved in 0.5 ml buffer (10 mM Tris, 10 mM $MgCl_2$, pH 8.5) and mixed with 5 µl (1.5 milliunits) of snake venom phosphodiesterase (SVPD). The mixture was incubated at 37° C in a thermally regulated cell and A_{260} was plotted against time. Oligonucleotide degradation was measured as function of increase in hyperchromicity.

The results of these experiments are shown in Table V, below. These results demonstrate that self-stabilized olignoucleotide phosphodiesters according to the invention are far more resistant to 3' exonucleotlytic degradation than either oligonucleotide phosphodiesters or oligonucleotide phosphodiesters having a non-complementary tail.

In addition to the testing described above, the oligonucleotides were also subjected to DNA Polymerase I 3'-exonuclease digestion. As shown in Figure 6 the non-self-stabilized oligonucleotides were digested to completion in 30 minutes, whereas the self-stabilized oligonucleotide with a 10 nucleotide self-complementary region was only partly digested over 30 minutes.

TABLE V
SVPD Half-Life Of Oligonucleotides

Supplementary Region Digestion	Half-Life For SVPD
Absent	75 seconds
10 nucleotides	950 seconds
mismatched	75 seconds

EXAMPLE 9

Nuclease Resistance of Self-Stabilized Oligonucleotide Phosphorothioates

To test the relative nuclease resistance of self-stabilized and non-self-stabilized oligonucleotide phosphorothioates, a DNA Polymerase I 3'-exonuclease activity assay was used, because of the slow degradation of oligonucleotide phosphorothioates by SVPD.

All oligonucleotides were labelled at the 5-end with gamma-^{32}P-ATP and kinase. To a solution of 40 pmole 5'-labelled oligonucleotide in 20 µl buffer (40 mM Tris HCl, pH 8.0,
10 mM $MgCl_2$, 5 mM DTT, 50 mM KCl, 50 µg/ml BSA), 5 units DNA polymerase I was added and incubated at 37° C. Aliquots of 4 µl were taken at 0, 30, 60, 120 minutes and were mixed with 6 µl stop solution (98% formamide, 10 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue). The samples were analyzed by 15% acrylamide gel (urea) and autoradiography.

The results are shown in Figure 7. Oligonucleotide phosphorothioates having no self-complementary region or only mismatched nucleotides (T_{10}) at the 3' end (as described in Example 8 for oligonucleotide phosphodiesters) were digested to almost 50% within 4 hours. The oligonucleotide phosphorothioate having a 10 nucleotide self-complementary region was undegraded after 4 hours. Oligonucleotide phosphorothioates having 6 or 4 nucleotide self-complementary regions were also found to be stable. These results demonstrate that self-stabilized oligonucleotide phosphorothioates are far more resistant to nucleolytic degradation than are non-self-stabilized oligonucleotide phosphorothioates.

EXAMPLE 10

Anti-Influenza Activity Of Modified Oligonucleotides

MDCK canine kidney cells were seeded in Minimum Essential Medium (MEM) with non-essential amino acids (GIBCO BRL, Grand Island, New York) with 5% fetal bovine serum (Hyclone Laboratories, Logen, Utah), 0.1% $NaHCO_3$, at a concentration of 4 x 10^5 cells/ml in 96 well tissue culture plates (Corning, Corning, New York) at 0.2 ml/well. The cells were incubated overnight to establish monolayers of cells. Growth medium was then removed and 0.1 ml of oligonucleotide at pre-selected concentrations in serum-free MEM containing 0.18% $NaHCO_3$ and

50 µg/ml gentamycin. This was done for each compound for each of 4 wells: 1 well as toxicity control and 3 wells as anti-viral tests. Three cell control wells and six virus control wells received 0.1 ml of serum-free MEM containing 0.18% $NaHCO_3$ and 50 µg/ml gentamycin. Within 10 minutes of addition of the oligonucleotide compounds, influenza A/NWS/33(H1N1) virus was added to each test well and virus control well in 0.1 ml MEM containing 0.18% $NaHCO_3$, 20 µg/ml trypsin, 2 µg/ml EDTA and

50 µg/ml gentamycin. Cell control and toxicity control wells received 0.1 ml of this same medium without virus.

Plates were incubated at 37° C in a humidified incubator with 5% CO_2, 95% air atmosphere. The cells were examined by microscopic observation for evidence of virus-specific cytopathic effect (CPE) and for morphological changes due to compound effect in non-infected toxicity controls. Virus CPE was graded on a scale of 0-4, with 4 being 95-100% CPE. The effective dose, 50% endpoint (ED_{50}) was calculated by regression analysis of the mean CPE grade at each concentration of the compound where activity was seen to bracket the CPE grade that was 50% of that seen in the virus controls. Visible changes in the morphology of cells in toxicity control wells were graded by microscopic observation, using a scale from no toxicity (0%) to complete destruction of the cells (100%) in 20% increments. The cytotoxic dose, 50%

endpoint (CD_{50}) was calculated by regression analysis of those toxicity grades bracketing the 50% endpoint, compared to the concentrations of compound used for those toxicity grades. A therapeutic index (TI) was calculated for each compound using the formula $TI = CD_{50}/ED_{50}$. These results are shown in Table VI, below.

TABLE VI
Anti-Influenza Activity Of Modified Oligonucleotides

CMPD	ED_{50} (μg/ml)	CD_{50} (μg/ml)	TI
A	Not Tested		
B	34.3	147	4.3
C	55	562	10
D	61	649	11
E	Not Tested		
F	178	261	1.5
G	147	649	4.4
H	59	562	9.5
I	45	750	17
J	175	750	4.3
K	22	825	38
L	32	422	13
M	23.7	383	16
Control[1]	440	422	<1.0

[1]Control is oligodeoxynucleotide phosphorothioate having the sequence 5' CAGAGCAAAATCATCAGAAGA 3'.

These results demonstrate that all of the preferred structural features of anti-influenza modified

oligonucleotides according to the invention, i.e., chimeric, hybrid, capped and self-stabilized features, are capable of improving the efficacy of antisense oligonucleotides in inhibiting influenza virus replication or propagation. These results further suggest that combinations of such structural features within an antisense oligonucleotide should have even greater efficacy.

EXAMPLE 11

Inhibition Of Various Strains Of Influenza Virus By Anti-influenza Modified Oligonucleotides

To test whether anti-fluenza modified oligonucleotides according to the invention can inhibit other strains of influenza virus, the experiment described in Example 10 was repeated, using compound M against various influenza strains. The influenza strains chosen for this study were the H1N1 strains A/NWS/33 and A/PR/8/34, the H3N2 strains A/Washington/897/80, A/Victoria/3/75 and A/Port Chalmers/1/73, and the H2N2 strain A2/Japan/305/57. The results were calculated as described in Example 10, and are shown in Table VII below.

TABLE VII
Effect Of Compound M On Various Strains of Influenza

Influenza Strain	ED_{50} (µg/ml)	CD_{50} (µg/ml)	TI
A/NWS/33	7.6	>100	>13
A/PR/8/34	>100	>100	?
A2/Japan/305/57	61	>100	>1.6
A/Washington/897/80	6	>100	17
A/Victoria/3/75	6.2	>100	>16
A/Port Chalmers/1/73	>100	>100	?

According to these results, compound M showed great efficacy in inhibiting three of the six strains of influenza tested and had some efficacy against a fourth strain. These results demonstrate that anti-influenza modified oligonucleotides according to the invention can be effective against multiple strains of influenza virus. To maximize the breadth of cross-strain efficacy, nucleotide sequences of various genes from several different influenza viruses can be compared and the most conserved nucleotide sequences can be used to prepare inhibitory oligonucleotides.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HYBRIDON, INC.

(ii) TITLE OF INVENTION: Novel Anti-Influenza Oligonucleotides

(iii) NUMBER OF SEQUENCES: 37

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Allegretti & Witcoff, Ltd.
(B) STREET: Ten South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
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(F) ZIP: 60606

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Michael S. Greenfield
(B) REGISTRATION NUMBER: 37,142
(C) REFERENCE/DOCKET NUMBER: 93,161-A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/715-1000
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2149 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCGAAAGCA GGCAAACCAT TTGAATGGAT GTCAATCCGA CTTTACTTTT CTTAAAAGTG 60

CCAGCACAAA ATGCTATAAG CACAACTTTC CCTTATACTG GAGACCCTCC TTACAGCCAT 120

GGGACAGGAA CAGGATACAC CATGGATACT GTCAACAGGA CACATCAGTA CTCAGAAAGG 180

GGAAGATGGA CAACAAACAC CGAAACTGGA GCACCGCAAC TCAACCCGAT TGATGGGCCA 240

CTGCCAGAAG ACAATGAACC AAGTGGTTAT GCCCAAACAG ATTGTGTATT GGAAGCAATG 300

GCCTTCCTTG AGGAATCCCA TCCTGGTATC TTTGAGACCT CGTGTCTTGA AACGATGGAG 360

GTTGTTCAGC AAACACGAGT GGACAAGCTG ACACAAGGCC GACAGACCTA TGACTGGACT 420

CTAAATAGGA ACCAGCCTGC TGCAACAGCA TTGGCCAACA CAATAGAAGT GTTCAGATCA 480

AATGGCCTCA CGGCCAATGA ATCCGGAAGG CTCATAGACT TCCTTAAGGA TGTAATGGAG 540

TCAATGAACA AAGAAGAAAT GGAGATCACA ACTCATTTTC AGAGAAAGAG ACGAGTGAGA 600

GACAATATGA CTAAGAAAAT GGTGACACAG AGAACAATAG GTAAAAGGAA GCAGAGATTG 660

AACAAAAGGA GTTATCTAAT TAGGGCATTG ACCCTGAACA CAATGACCAA AGATGCTGAG 720

AGAGGGAAGC TAAAACGGAG AGCAATTGCA ACCCCAGGGA TGCAAATAAG GGGGTTTGTA 780

TACTTTGTTG AGACACTAGC AAGGAGTATA TGTGAGAAAC TTGAACAATC AGGATTGCCA 840

GTTGGAGGCA ATGAGAAGAA AGCAAAGTTG GCAAATGTTG TAAGGAAGAT GATGACCAAT 900

TCTCAGGACA CTGAAATTTC TTTCACATCA CTGGAGATAA CACCAAATGG AACGAAAATC 960

AGAACCCTCG GATGTTTTTG GCCATGATCA CATATATAAC CAGAAATCAG CCCGAATGGT 1020

TCAGAAATGT TCTAAGTATT GCTCCAATAA TGTTCTCAAA CAAAATGGCG AGACTGGGAA 1080

AGGGGTACAT GTTTGAGAGC AAGAGTATTA AAATTAGAAC TCAAATACCT GCAGAAATGC 1140

TAGCAAGCAT CGATTTGAAA TACTTCAATG ATTCAACTAG AAAGAAGATT GAAAAAATCC 1200

GGCCGCTCTT AATAGATGGG ACTGCATCAT TGAGCCCTGG AATGATGATG GGCATGTTCA 1260

ATATGTTAAG TACTGTATTA GGCGTCTCCA TCCTGAATCT TGGACAAAAG AGACACACCA 1320

AGACTACTTA CTGGTGGGAT GGTCTTCAAT CTTCTGATGA TTTTGCTCTG ATTGTCAATG 1380

CACCCAATCA TGAAGGGATT CAAGCCGGAG TCAACAGGTT TTATCGAACC TGTAAGCTAC 1440

TTGGAATTAA TATGAGCAAG AAAAAGTCTT ACATAAACAG AACAGGTACA TTTGAATTCA 1500

CAAGTTTTTT CTATCGTTAT GGGTTTGTTG CCAATTTCAG CATGGAGCTT CCCAGCTTTG 1560

GGGTGTCTGG GATCAACGAG TCTGCGGACA TGAGTATTGG AGTTACTGTC ATCAAAAACA 1620

ATATGATAAA CAATGATCTT GGTCCAGCAA CCGCTCAAAT GGCCCTTCAG CTGTTCATCA 1680

AAGATTACAG GTACACGTAC CGCTGCCATA GAGGTGACAC ACAAATACAA ACCCGAAGAT 1740

CATTTGAAAT AAAGAAACTG TGGGAGCAAA CCCATTCCAA AGCTGGACTG CTGGTCTCCG 1800

ACGGAGGCCC AAATTTATAC AACATTAGAA ATCTCCACAT TCCTGAAGTC TGCTTGAAAT 1860

GGGAATTAAT GGATGAGGAT TACCAGGGGC GTTTATGCAA CCCACTGAAC CCATTTGTCA 1920

ACCATAAAGA CATTGAATCA GTGAACAATG CAGTGATAAT GCCAGCACAT GGTCCAGCCA 1980

AAAACATGGA GTATGATGCT GTTGCAACAA CACACTCCTG GATCCCCAAA AGAAATCGAT 2040

CCATCTTGAA TACAAGCCAA AGAGGAATAC TTGAAGATGA ACAAATGTAC CAAAAGTGCT 2100

GCAACTTATT TGAAAAATTC TTCCCCAGCA GTTCATACAG AAGACCAGT 2149

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGAGCAAAAT CATCAGAAG 19

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGAGCAAAAT CATCAGAAG 19

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAGCAAAATC ATCAGAA 17

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGCAAAATC ATCAGAA 17

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGAGCAAAA TCATCAGAAG A 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CAGAGCAAAA TCATCAGAAG ATTCTGATGA 30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTTTCCATAT TGAATATAAT 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ACATCCATTC AAATGGTTTG 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCTTCCATTT TGGATCAGTA 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCCTTCATTT TGGTTGTTTT 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GACGCCATGA TTTTGATGTC 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGATTCATTT TAAACCCCTG 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGACTCATCT TTCAATATCT 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GATAGAGAGA ACGTACGTTT 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCTGATAGGC CTGCAAATTT 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGATCCATTA TGTCTTTGTC 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CATGTCGGTT AGGTAACGCG 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCAATCTACC TGAAAGCTTG 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGCAGTATGT CCTGGAAGAG 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAAACGACCT TGTTTCTACT 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAAAATGCCT TGTTCCTACT 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAAAGTACCT TGTTTCTACT 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AAAACACCCT TGTTTCTACT 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

AAAATACCCT TGTTTCTACT 20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AAAAACTCCT TGTTTCTACT 20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AAAACTACCT TGTTTCTACT 20

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AAAACACCCT TGTTTCTACT 20

We claim:

1. An anti-influenza modified oligonucleotide comprising a nucleotide sequence that is complementary to an essential nucleic acid sequence of influenza virus, wherein the oligonucleotide is a mixed backbone chimeric oligonucleotide comprising a phosphorothioate or phosphorodithioate region and an alkylphosphonate region.

2. The oligonucleotide according to claim 1, wherein an alkylphosphonate region is at or near the 5' or 3' end of the oligonucleotide.

3. The oligonucleotide according to claim 1, wherein an alkylphosphonate region comprises from about 2 to about 10 contiguous nucleotides connected by alkylphosphonate linkages.

4. The oligonucleotide according to claim 2, wherein an alkylphosphonate region comprises from about 2 to about 10 contiguous nucleotides connected by alkylphosphonate linkages.

5. The oligonucleotide according to claim 1, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

6. The oligonucleotide according to claim 2, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

7. The oligonucleotide according to claim 3, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

8. The oligonucleotide according to claim 4, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

9. An anti-influenza modified oligonucleotide comprising a nucleotide sequence that is complementary to an essential nucleic acid sequence of influenza virus, wherein the oligonucleotide is a mixed backbone chimeric oligonucleotide comprising a phosphorothioate or phosphorodithioate region and an alkylphosphonothioate region.

10. The oligonucleotide according to claim 10, wherein an alkylphosphonothioate region is at or near the 5' or 3' end of the oligonucleotide.

11. The oligonucleotide according to claim 9, wherein an alkylphosphonothioate region comprises from about 2 to about 10 contiguous nucleotides connected by alkylphosphonothioate linkages.

12. The oligonucleotide according to claim 10, wherein an alkylphosphonothioate region comprises from about 2 to about 10 contiguous nucleotides connected by alkylphosphonothioate linkages.

13. The oligonucleotide according to claim 9, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

14. The oligonucleotide according to claim 10, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

15. The oligonucleotide according to claim 11, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

16. The oligonucleotide according to claim 12, wherein a phosphorothioate or phosphorodithioate region comprises from about 3 to about 100 contiguous nucleotides connected by phosphorothioate or phosphorodithioate linkages.

17. An anti-influenza modified oligonucleotide comprising a nucleotide sequence that is complementary to an essential nucleic acid sequence of influenza virus, wherein the oligonucleotide is a hybrid oligonucleotide comprising a deoxyribonucleotide region and a ribonucleotide region.

18. The oligonucleotide according to claim 17, further comprising from about 1 to about all phosphorothioate or phosphorodithioate internucleotide linkages.

19. The oligonucleotide according to claim 17, wherein a ribonucleotide region is at or near the 5' or 3' end of the oligonucleotide.

20. The oligonucleotide according to claim 17, wherein a ribonucleotide region comprises from about 2 to about 100 contiguous ribonucleotides.

21. The oligonucleotide according to claim 18, wherein a ribonucleotide region comprises from about 2 to about 100 contiguous ribonucleotides.

22. The oligonucleotide according to claim 19, wherein a ribonucleotide region comprises from about 2 to about 100 contiguous ribonucleotides.

23. The oligonucleotide according to claim 17, wherein a deoxyribonucleotide region comprises from about 0 to about 100 deoxyribonucleotides.

24. The oligonucleotide according to claim 18, wherein a deoxyribonucleotide region comprises from about 0 to about 100 deoxyribonucleotides.

25. The oligonucleotide according to claim 19, wherein a deoxyribonucleotide region comprises from about 0 to about 100 deoxyribonucleotides.

26. The oligonucleotide according to claim 20, wherein a deoxyribonucleotide region comprises from about 0 to about 100 deoxyribonucleotides.

27. The oligonucleotide according to claim 21, wherein a deoxyribonucleotide region comprises from about 0 to about 100 deoxyribonucleotides.

28. The oligonucleotide according to claim 22, wherein a deoxyribonucleotide region comprises from about 0 to about 100 deoxyribonucleotides.

29. An anti-influenza modified oligonucleotide comprising a nucleotide sequence that is complementary to an essential nucleic acid sequence of influenza virus, wherein the oligonucleotide has at its 5' and/or 3' end a nuclease resistance conferring cap structure selected from the group consisting of the cap structures shown in Figure 4 and lower alkyl (C1-C12) or alcohol groups, and wherein the oligonucleotide has from 1 to about all modified internucleotide linkages selected from the group consisting of phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, sulfone, phosphorothioate and phosphorodithioate linkages.

30. An anti-influenza modified oligonucleotide comprising an anti-influenza hybridizing region and a self-complementary region.

31. The oligonucleotide according to claim 30, wherein the influenza hybridizing region comprises from about 6 to about 100 nucleotides that are complementary to an essential nucleic acid sequence of influenza virus.

32. The oligonucleotide according to claim 30, wherein the self-complementary region comprises from about 4 to about 50 nucleotides that form intramolecular base pairs.

33. The oligonucleotide according to claim 30, wherein a self-complementary region is at or near the 5' or 3' end of the oligonucleotide.

34. The oligonucleotide according to claim 31, wherein a self-complementary region is at or near the 5' or 3' end of the oligonucleotide.

35. The oligonucleotide according to claim 32, wherein a self-complementary region is at or near the 5' or 3' end of the oligonucleotide.

36. The oligonucleotide according to claim 30, wherein from about 2 to about all nucleotides are coupled by phosphorothioate and/or phosphorodithioate internucleotide linkages.

37. The oligonucleotide according to claim 31, wherein from about 2 to about all nucleotides are coupled by phosphorothioate and/or phosphorodithioate internucleotide linkages.

38. The oligonucleotide according to claim 32, wherein from about 2 to about all nucleotides are coupled by phosphorothioate and/or phosphorodithioate internucleotide linkages.

39. The oligonucleotide according to claim 33, wherein from about 2 to about all nucleotides are coupled by phosphorothioate and/or phosphorodithioate internucleotide linkages.

40. The oligonucleotide according to claim 34, wherein from about 2 to about all nucleotides are coupled by phosphorothioate and/or phosphorodithioate internucleotide linkages.

41. The oligonucleotide according to claim 35, wherein from about 2 to about all nucleotides are coupled by phosphorothioate and/or phosphorodithioate internucleotide linkages.

42. The oligonucleotide according to claim 1, wherein the essential nucleic acid sequence is selected from the group consisting of influenza polymerase 3 gene, influenza polymerase 1 gene, influenza polymerase 2 gene, influenza hemaglutin gene, influenza nucleoprotein gene, influenza neuraminidase gene, influenza matrix protein gene, influenza left or right splice junctions of segments 7 or 8, influenza splice branch of segment 8 and influenza packaging sequences of segment 1, 2, 3, 4, 5, 6, 7 or 8.

43. The oligonucleotide according to claim 9, wherein the essential nucleic acid sequence is selected from the group consisting of influenza polymerase 3 gene, influenza polymerase 1 gene, influenza polymerase 2 gene, influenza hemaglutin gene, influenza nucleoprotein gene, influenza neuraminidase gene, influenza matrix protein gene, influenza left or right splice junctions of segments 7 or 8, influenza splice branch of segment 8 and influenza packaging sequences of segment 1, 2, 3, 4, 5, 6, 7 or 8.

44. The oligonucleotide according to claim 17, wherein the essential nucleic acid sequence is selected from the group consisting of influenza polymerase 3 gene, influenza polymerase 1 gene, influenza polymerase 2 gene, influenza hemaglutin gene, influenza nucleoprotein gene, influenza neuraminidase gene, influenza matrix protein gene, influenza left or right splice junctions of segments 7 or 8, influenza splice branch of segment 8 and influenza packaging sequences of segment 1, 2, 3, 4, 5, 6, 7 or 8.

45. The oligonucleotide according to claim 29, wherein the essential nucleic acid sequence is selected from the group consisting of influenza polymerase 3 gene, influenza polymerase 1 gene, influenza polymerase 2 gene, influenza hemaglutin gene, influenza nucleoprotein gene, influenza neuraminidase gene, influenza matrix protein gene, influenza left or right splice junctions of segments 7 or 8, influenza splice branch of segment 8 and influenza packaging sequences of segment 1, 2, 3, 4, 5, 6, 7 or 8.

46. The oligonucleotide according to claim 31, wherein the essential nucleic acid sequence is selected from the group consisting of influenza polymerase 3 gene, influenza polymerase 1 gene, influenza polymerase 2 gene, influenza hemaglutin gene, influenza nucleoprotein gene, influenza

neuraminidase gene, influenza matrix protein gene, influenza left or right splice junctions of segments 7 or 8, influenza splice branch of segment 8 and influenza packaging sequences of segment 1, 2, 3, 4, 5, 6, 7 or 8.

1 AGCGAAAGCATGGCAAACCATTGAATGGAT GTCAATCCGA CTTTACTTTT
51 CTTAAAAGTG CCAGCACAAA ATGCTATAAG CACAACTTTC CCTTATACTG
101 GAGACCCTCC TTACAGCCAT GGGACAGGAA CAGGATACAC CATGGATACT
151 GTCAACAGGA CACATCAGTA CTCAGAAAGG GGAAGATGGA CAACAAACAC
201 CGAAACTGGA GCACCGCAAC TGAACCCGAT TGATGGGCCA CTGCCAGAAG
251 ACAATGAACC AAGTGGTTAT GCCCAAACAG ATTGTGTATT GGAAGCAATG
301 GCCTTCCTTG AGGAATCCCA TCCTGGTATC TTTGAGACCT CGTGTCTTGA
351 AACGATGGAG GTTGTTCAGC AAACACGAGT GGACAAGCTG ACACAAGGCC
401 GACAGACCTA TGACTGGACT CTAAATAGGA ACCAGCCTGC TGCAACAGCA
451 TTGGCCAACA CAATAGAAGT GTTCAGATCA AATGGCCTCA CGGCCAATGA
501 ATCCGGAAGG CTCATAGACT TCCTTAAGGA TGTAATGGAG TCAATGAACA
551 AAGAAGAAAT GGAGATCACA ACTCATTTTC AGAGAAAGAG ACGAGTGAGA
601 GACAATATGA CTAAGAAAAT GGTGACACAG AGAACAATAG GTAAAAGGAA
651 GCAGAGATTG AACAAAAGGA GTTATCTAAT TAGGGCATTG ACCCTGAACA
701 CAATGACCAA AGATGCTGAG AGAGGGAAGC TAAAACGGAG AGCAATTGCA
751 ACCCCAGGGA TGCAAATAAG GGGGTTTGTA TACTTTGTTG AGACACTAGC
801 AAGGAGTATA TGTGAGAAAC TTGAACAATC AGGATTGCCA GTTGGAGGCA
851 ATGAGAAGAA AGCAAAGTTG GCAAATGTTG TAAGGAAGAT GATGACCAAT
901 TCTCAGGACA CTGAAATTTC TTTCACCATC ACTGGAGATA ACACCAAATG
951 GAACGAAAAT CAGAACCCTC GGATGTTTTT GGCCATGATC ACATATATAA
1001 CCAGAAATCA GCCCGAATGG TTCAGAAATG TTCTAAGTAT TGCTCCAATA
1051 ATGTTCTCAA ACAAAATGGC GAGACTGGGA AAGGGGTACA TGTTTGAGAG

Fig. 1

1101 CAAGAGTATT AAAATTAGAA CTCAAATACC TGCAGAAATG CTAGCAAGCA
1151 TCGATTTGAA ATACTTCAAT GATTCAACTA GAAAGAAGAT TGAAAAAATC
1201 CGGCCGCTCT TAATAGATGG GACTGCATCA TTGAGCCCTG GAATGATGAT
1251 GGGCATGTTC AATATGTTAA GTACTGTATT AGGCGTCTCC ATCCTGAATC
1301 TTGGACAAAA GAGACACACC AAGACTACTT ACTGGTGGGA TGGTCTTCAA
1351 TCTTCTGATG ATTTTGCTCT GATTGTGAAT GCACCCAATC ATGAAGGGAT
1401 TCAAGCCGGA GTCAACAGGT TTTATCGAAC CTGTAAGCTA CTTGGAATTA
1451 ATATGAGCAA GAAAAAGTCT TACATAAACA GAACAGGTAC ATTTGAATTC
1501 ACAAGTTTTT TCTATCGTTA TGGGTTTGTT GCCAATTTCA GCATGGAGCT
1551 TCCCAGCTTT GGGGTGTCTG GGATCAACGA GTCTGCGGAC ATGAGTATTG
1601 GAGTTACTGT CATCAAAAAC AATATGATAA ACAATGATCT TGGTCCAGCA
1651 ACCGCTCAAA TGGCCCTTCA GCTGTTCATC AAAGATTACA GGTACACGTA
1701 CCGGTGCCAT AGAGGTGACA CACAAATACA AACCCGAAGA TCATTTGAAA
1751 TAAAGAAACT GTGGGAGCAA ACCCATTCCA AAGCTGGACT GCTGGTCTCC
1801 GACGGAGGCC CAAATTTATA CAACATTAGA AATCTCCACA TTCCTGAAGT
1851 CTGCTTGAAA TGGGAATTAA TGGATGAGGA TTACCAGGGG CGTTTATGCA
1901 ACCCACTGAA CCCATTTGTC AACCATAAAG ACATTGAATC AGTGAACAAT
1951 GCAGTGATAA TGCCAGCACA TGGTCCAGCC AAAAACATGG AGTATGATGC
2001 TGTTGCAACA ACACACTCCT GGATCCCCAA AAGAAATCGA TCCATCTTGA
2051 ATACAAGCCA AAGAGGAATA CTTGAAGATG AACAAATGTA CCAAAAGTGC
2101 TGCAACTTAT TTGAAAAATT CTTCCCCAGC AGTTCATACA GAAGACCAGT

Fig. 1
(cont'd)

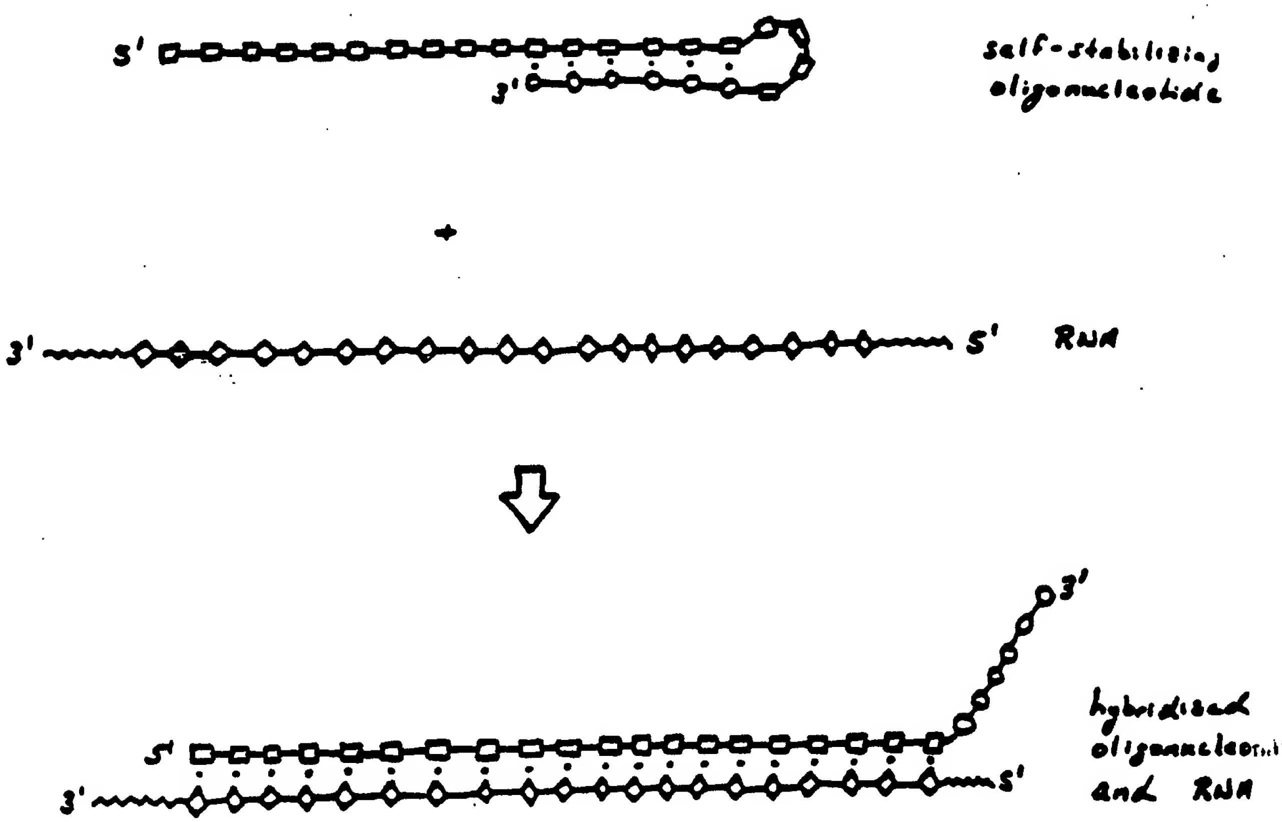

5'
3'
self-stabilizing oligonucleotide
+
3'
5'
RNA
3'
5'
3'
5'
hybridized oligonucleotide and RNA

Fig. 2

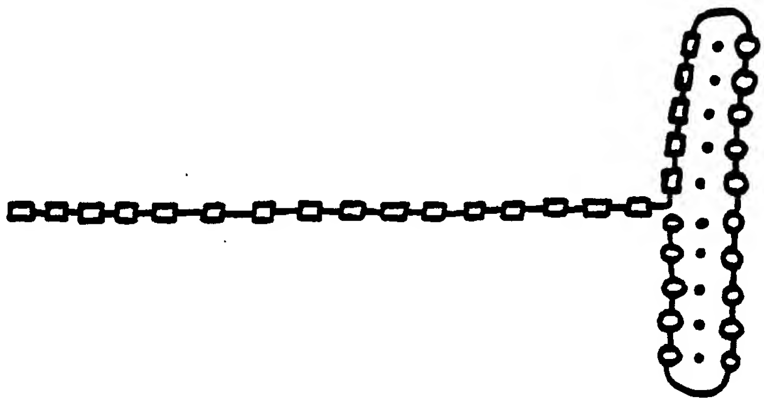
5'


Fig. 3

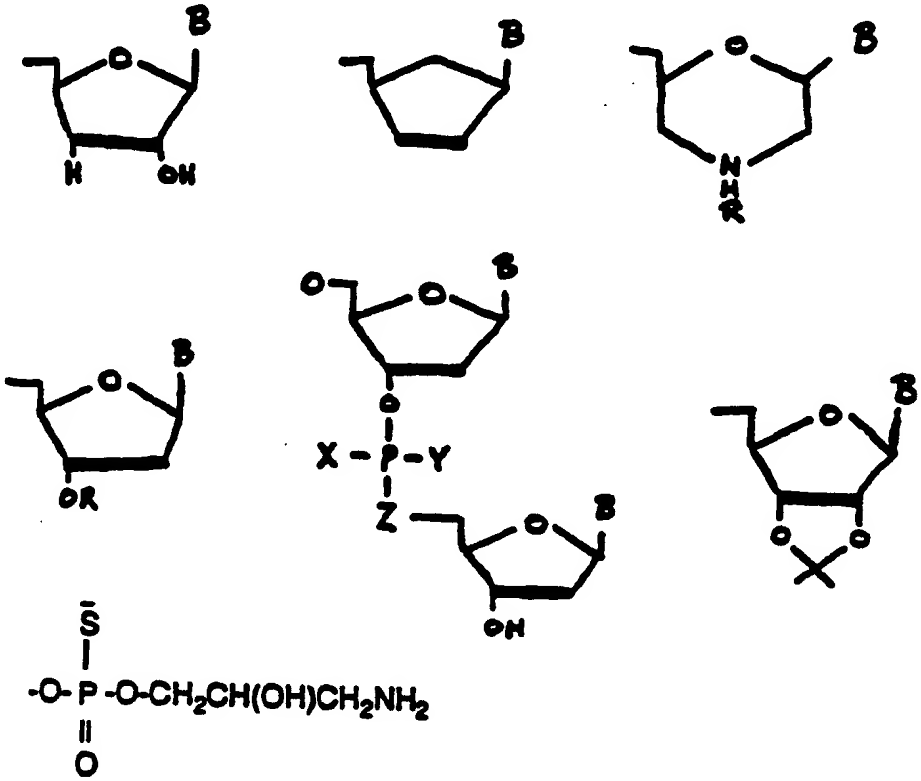


Fig. 4

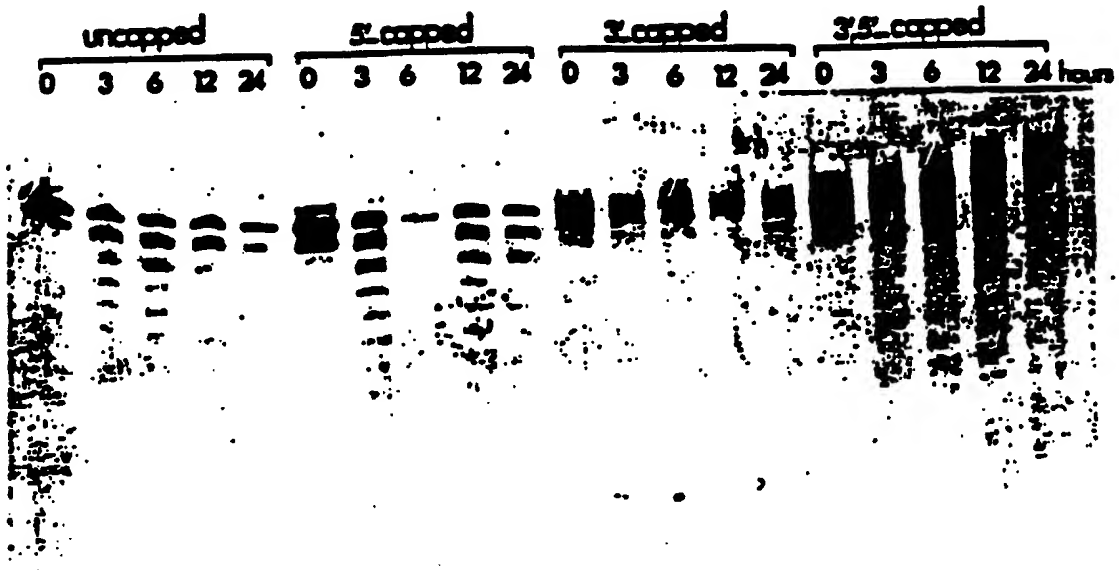

uncapped
5'-capped
3'-capped
3',5'-capped
0 3 6 12 24
0 3 6 12 24
0 3 6 12 24
0 3 6 12 24 hours

Fig. 5

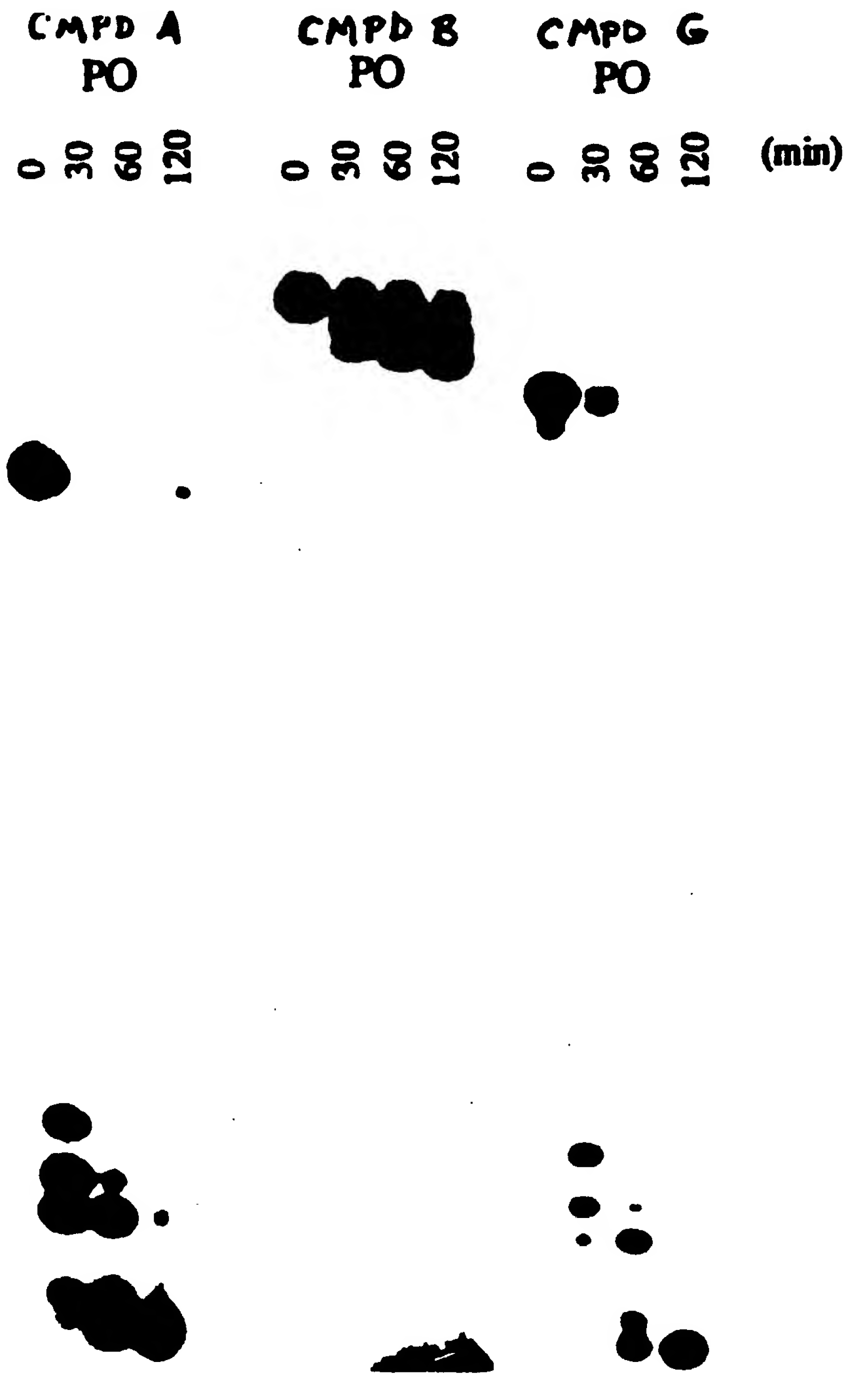


Fig. 6

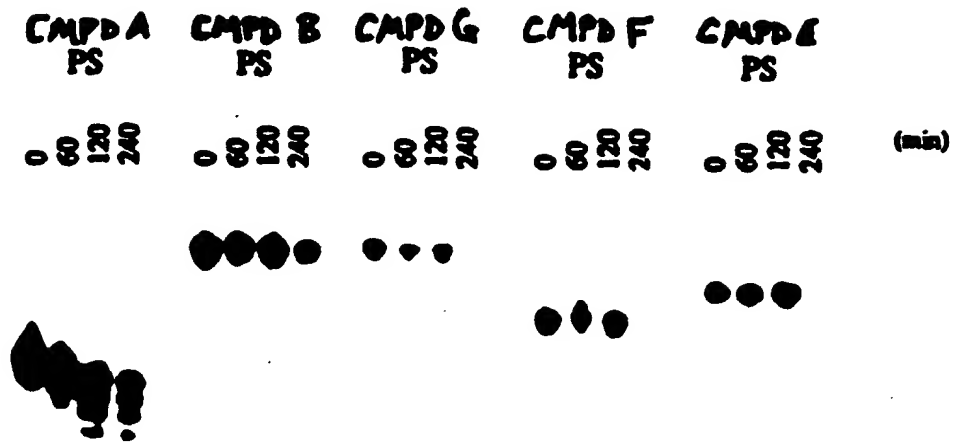


Fig. 7